In quantitative polymerase chain reaction analysis, the spike-in control is used to adjust for differences in extraction efficiency between samples (5), and the intersample deviation of Cel-miR-39 measurements is usually less than 1 cycle. However, immediately after administration of the heparin bolus, the detectability of Cel-miR-39 decreases by approximately 3 cycles. This effect is confined to the first hours after heparin dosing and directly related to the half-life of heparin in the circulation. Thus, heparin could have interfered with the quantitation of miRNA after transcoronary ablation of septal hypertrophy. The accompanying editorial noted "the stunning precocity of elevation in the peripheral circulation of miR-1 and miR-133: only 15 min" (6). If baseline blood samples were taken before administration of heparin, then the rapid increase may at least in part be explained by the effect of heparin on the normalization control. If the baseline samples were taken after the heparin bolus, then the reference samples are not suitable for measurements of miRNA after the first hour post-dose. Furthermore, a significant increase in plasma miR-21 levels was previously observed after thigh cuff–induced ischemia/reperfusion injury (7). Plasma miR-21 levels are also affected by antiplatelet medication (8). Thus, miR-21 may not be a suitable control in this setting. Describing the nature and timing of treatments administered in miRNA biomarker studies is necessary to facilitate interpretation of data and prevent confounding by treatment effects.

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Reply

Effects of Heparin on Temporal MicroRNA Profiles

We read with great interest the letter by Dr. Mayr and colleagues regarding our work and the important topic of the effects of heparin on temporal microRNA (miRNA) profiles and the best timing of miRNA measurement after administration of heparin. Dr. Mayr and colleagues were recently able to show that both the timing of blood sampling relative to heparin dosing and the normalization procedure are critical for reliable miRNA measurements in patients receiving intravenous heparin (2,3). At this point, it can clearly be stated that heparin is not the only confounder in the setting of miRNA measurements; several factors, including different isolation protocols, blood sample type (plasma or serum), and inflammation-driven shifts in hematopoietic compartments after myocardial infarction, appear to further affect the detection of cell-free (truly circulating) miRNA (4).

With reference to the letter, the baseline blood samples in our study (1) were taken before administration of heparin. Therefore, we have a true control for the comparison of miRNA concentrations after transcoronary ablation of septal hypertrophy. The transcoronary ablation of septal hypertrophy procedure itself was performed after administration of heparin (bolus 5,000 IU heparin). The miRNAs were isolated from serum samples before miRNA isolation and spiking with native cel-miR-39. The samples were treated with heparinase to minimize the influence of heparin on the miRNA measurements. Of course, we cannot entirely exclude the further existence of an effect of heparin, but pre-study tests using the heparinase protocol showed comparable results for miRNA concentrations with and without heparin. Furthermore, our results clearly show a steep increase in miR-1 and miR-133a levels (approximately a 30-fold change) within 60 min after induction of myocardial infarction. Even if heparin were to cause a 30% difference in miRNA concentrations, the difference compared with miRNA concentrations at 15 min would still be significant.

The patients in our study were without antiplatelet medication. Therefore, we can exclude the interference of this medication with the measurement of miR-21 concentrations. Nevertheless, miR-21 is known to be up-regulated in the presence of cardiac hypertrophy. Thus, an influence on this subset cannot be entirely excluded.

Although methodological issues may slightly interfere with the final miRNA measurements, our results add important information to this new field, and miRNAs may well have a future as biomarkers for myocardial ischemia.

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Limits of Mendelian Randomization Analyses in Selection of Secretory Phospholipase A_2-IIA as a Valid Therapeutic Target for Prevention of Cardiovascular Disease

Holmes et al. (1) investigated the association between secretory phospholipase A_2-IIA ($sPLA_2$-IIA) as a potential therapeutic target for prevention of cardiovascular disease, using observational studies between the *PLA2G2A* rs11573156 variant and cardiovascular events, and deductions from published data with the pan-$sPLA_2$ inhibitor varespladib methyl. The validity of the analysis by Holmes et al. remains unclear due to inaccurate summary of data, incorrect assumptions related to the biology of $sPLA_2$-IIA, and the pharmacological effects of varespladib (2). For brevity, the major issues include the following:

1. No reporting of the absolute values for $sPLA_2$-IIA levels and activity, which is important because of variable results with different analytical methods and marked differences in $sPLA_2$ levels that result from the acute phase reaction in patients with acute coronary syndrome versus patients with stable coronary heart disease;
2. Use of total $sPLA_2$ activity as a surrogate for $sPLA_2$-IIA activity;
3. Investigation of the *PLA2G2A* rs11573156 variant with messenger RNA expression even though none of the variants illustrated in Figure 2 included the variant as a genetic tool in the current analysis;
4. A marginally significant correlation between *PLA2G2A* rs11573156 variant and $sPLA_2$ activity despite higher correlations between $sPLA_2$-IIA mass and $sPLA_2$ activity reported in Online Figure 2;
5. Reporting of percentage changes in $sPLA_2$ levels and $sPLA_2$ activity when absolute changes may be more important, particularly because of wide differences in baseline $sPLA_2$-IIA levels in different cohorts;
6. A lack of clear presentation from the original studies of the reported effects of varespladib on $sPLA_2$-IIA mass, and in fact, the table understates the effect of varespladib 500 mg/day on $sPLA_2$-IIA mass by more than 50%, whereas the reduction is 80%; the authors concluded incorrectly that the homozygous rs11573156C allele resulted in a reduction in $sPLA_2$-IIA mass "similar" to the effect of varespladib;
7. Biomarker effects of $sPLA_2$ in randomized clinical trials with >500 subjects should have included only the FRANCIS (Fewer Recurrent Acute Coronary Events With Near-Term Cardiovascular Inflammation Suppression)-ACS trial on the basis of the sample size requirement, but 3 trials are reported;
8. Biomarker effects should have been reported from the PLASMA I (Phospholipase Levels And Serological Markers of Atherosclerosis) and PLASMA II (Phospholipase Levels And Serological Markers of Atherosclerosis II) trials because these trials reported results from clinically stable patients, whereas the FRANCIS-ACS trial mandated a change in statin therapy for all patients to atorvastatin 80 mg daily regardless of their prior statin regimen;
9. Varespladib is a pan-$sPLA_2$ inhibitor with similar efficacy in lowering groups IIA and X $sPLA_2$ with somehow lower potency against group V, despite the incorrect data cited in this report (3).

Moreover, the use of Mendelian randomization studies to deduce pharmacological effects does not account for the properties of the specific inhibitor. Specifically, varespladib is hydrophilic and may not penetrate into vascular tissues with sufficient potency to reduce intracellular effects versus the consistent effects on plasma biomarkers. Also, because varespladib methyl inhibits $sPLA_2$-X and a recent report by Ait-Oufella et al. (4) demonstrated that overexpression of $sPLA_2$-X is atheroprotective, nonspecific effects of varespladib as a pan inhibitor may have positive and negative effects. In addition, preliminary results from the VISTA-16 trial (Evaluation of Safety and Efficacy of Short-term A-002 Treatment in Subjects With Acute Coronary Syndrome) indicate an increase in myocardial infarctions, so the effect of varespladib was harmful (5). As discussed previously, proinflammatory pathways are redundant, and multiple anti-inflammatory pathways modulate inflammatory responses.

In conclusion, careful review of primary data and cautious conclusions must be considered in these pharmacogenetic analyses (6).

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Please note: Dr. Rosenson has served as an advisory board member for Aegerion, Amgen, F. Hoffman LaRoche, GlaxoSmithKline, Janssen, LipoScience, Novartis, Regeneron, Sanofi; served as a consultant for Novartis and Sanofi; has received